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Kynurenine Levels in Patients with Schizophrenia with Elevated Anti-Gliadin Immunoglobulin G Antibodies

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Abstract

Objective—Several studies have reported an association between non-celiac gluten sensitivity and schizophrenia. Immune and kynurenine pathways have also been implicated in the pathophysiology of schizophrenia, and certain proinflammatory immune mediators may increase kynurenine (KYN) and reduce tryptophan (TRP) levels.

Methods—We thus measured serum anti-gliadin IgG, KYN and TRP in 950 patients with schizophrenia. Patients with antibody level at the 90th percentile or higher of control participants (21.9% of all patients) were classified as having elevated anti-gliadin IgG. Independent *t*-tests and linear regression models were used to compare TRP, KYN and KYN-TRP ratio (indicator of TRP metabolism) between patients with, and those without elevated anti-gliadin IgG. The correlation between anti-gliadin IgG and TRP, KYN and the ratio was also evaluated in the patients.

Results—KYN and KYN-TRP ratio were higher in patients with elevated anti-gliadin IgG (geometric mean 2.65 mmol/l, SD = 0.25 vs. 2.25mmol/l, SD = 0.23, $p < 0.001$ and 0.05, SD = 0.26 vs. 0.04, SD = 0.25, $p = 0.001$ respectively), findings robust to adjustment for potential demographic and clinical confounders. Anti-gliadin IgG correlated with KYN and KYN-TRP ratio in unadjusted analysis ($r = 0.12$, $p < 0.001$; $r = 0.11$, $p = 0.002$). TRP did not differ between the 2 groups and did not correlate with anti-gliadin IgG.

Conclusions—Our results connect non-celiac gluten sensitivity with the kynurenine pathway of tryptophan metabolism in psychotic illness and hint towards potential individualized treatment targets.

Keywords

kynurenine; tryptophan; schizophrenia; antigliadin Ig antibodies; non-celiac gluten sensitivity

Introduction

Gliadin is one of the constituent proteins of gluten, and both class A and class G anti-gliadin immunoglobulin (IgA and IgG) antibodies have been reported to be more prevalent and elevated in patients with schizophrenia when compared with healthy controls (1–4). Furthermore, the finding of an association between high levels of maternal IgG anti-gliadin during pregnancy and increased risk of non-affective psychosis in the offspring suggests the involvement of IgG anti-gliadin in the pathogenesis of psychosis (5). The association of elevated anti-gliadin antibodies with schizophrenia parallels the observation from epidemiologic studies that celiac disease, an immune-mediated disorder (which is also associated with antibodies to gliadin), might confer an increased risk of schizophrenia (6–8). However, the anti-gliadin immune response in schizophrenia has been noted to be different from that in celiac disease in that most patients with schizophrenia who are also seropositive for anti-gliadin do not express anti-transglutaminase and anti-deamidated gliadin peptide

IgG, biomarkers specific for celiac disease (9). Non-celiac gluten sensitivity is the term frequently used to describe individuals with elevated anti-gliadin IgG who do not have histological or serological features of celiac disease. Non-celiac gluten sensitivity is now recognized as distinct a clinical entity (10) that, also appears to be associated with psychiatric and neurologic manifestations (11–13).

The increased seroprevalence and serointensity of anti-gliadin antibodies seen in schizophrenia is possibly a reflection of abnormal immune function with accompanying imbalance in modulation of inflammation. Abnormal immune system function, including elevated inflammation, has been reported in schizophrenia on a relatively consistent basis (14, 15). Some of the lines of evidence in support of a neuroinflammation hypothesis of schizophrenia include a) levels of blood and cerebrospinal fluid (CSF) proinflammatory cytokines are elevated in patients with schizophrenia (16); b) maternal exposure to infections and the resulting inflammatory responses may elevate the risk of schizophrenia in the offspring during pregnancy (17, 18); c) the presence of an autoimmune disease in an individual or a relative has been reported to be associated with an increased risk of schizophrenia in the individual (19); d) genes in the major histocompatibility complex have been identified as risk factors for schizophrenia (20) and they, along with IgG genes, also contribute to anti-gliadin antibody responsiveness (21); and e) medications with anti-inflammatory properties such as minocycline, aspirin and cyclooxygenase-2 inhibitors have demonstrated potential utility in treating symptoms of schizophrenia (22).

Kynurenine (KYN) is produced by degradation of tryptophan (TRP) and is a precursor of certain neuroactive metabolites. The breakdown of TRP into KYN is catalyzed by both indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO). TRP is also a precursor of serotonin and its conversion to KYN and other downstream compounds is under the control of an enzymatic cascade called the TRP-KYN pathway. Downstream compounds of the KYN pathway include kynurenic acid (KYNA) and quinolinic acid (QUIN). KYN levels are elevated in the brain and CSF of patients with schizophrenia (23, 24).

Furthermore, the findings of: a) elevated prefrontal cortical and CSF levels of KYNA in patients with schizophrenia (25, 26); b) KYNA as a naturally occurring N-methyl D-aspartate (NMDA) glutamate receptor antagonist (27); c) psychotomimetic drugs phencyclidine and ketamine (both of which induce schizophrenia-like symptoms in normal individuals) blocking NMDA glutamate receptors (28) and reduced NMDA glutamate neurotransmission in schizophrenia (29) led to the introduction of the KYNA hypothesis of schizophrenia (30).

The TRP-KYN pathway is partially controlled by the immune system, and interferon- γ and other proinflammatory cytokines are among the most potent stimulators of IDO. TDO on the other hand, is upregulated mainly by cortisol (31) which has been reported to be elevated in patients with schizophrenia (32). Therefore, TRP conversion to KYN is enhanced in the presence of inflammation and raised cortisol level. To our knowledge, no study has evaluated the association of anti-gliadin IgG with either TRP or KYN in patients with schizophrenia. We therefore aimed to evaluate TRP and KYN serum levels in patients with schizophrenia with elevated anti-gliadin IgG versus those without elevated anti-gliadin IgG and to correlate anti-gliadin IgG levels with TRP and KYN levels. We also aimed to evaluate

the KYN-TRP ratio (an indicator of TRP metabolism) and correlate anti-gliadin IgG levels with this ratio in the two patient groups. We hypothesized that TRP levels are lower while KYN levels and KYN-TRP ratio are higher in patients with elevated anti-gliadin IgG and that anti-gliadin IgG is correlated with KYN levels and KYN-TRP ratio in the same patient group.

Methods

Participants

Details of how subjects were recruited into this study and the characteristics of the subjects have previously been described (3). In brief, 950 patients with schizophrenia (as defined by DSM-IV) aged 18–60 years were recruited in Munich, Germany. The Structured Clinical Interview for DSM-IV (33) and Positive and Negative Syndrome Scale (PANSS) (34) were administered on all the patients, all of whom were recruited from both inpatient and outpatient clinics.

Informed consent was obtained from all participants after the study procedures were explained in detail. The local ethics committee of Ludwig Maximilians University, Munich, Germany approved the study and it was considered exempt by the Institutional Review Board of the University of Maryland School of Medicine Baltimore, MD, USA.

Blood collection, processing and serological analysis

Blood was drawn into EDTA containing monovettes at any time of the day, transferred to the laboratory and centrifuged immediately after arrival (4°C, 10min, 3000rpm) but no later than 3 hours after the time of blood draw. Samples were stored at –80°C. Blood collection took place from April 1998 to May 2010.

A previously described solid-enzyme immunoassay method (2) was used to measure serum anti-gliadin IgG in all participants. The commercially available ELISA kits were obtained from IBL International, Hamburg, Germany. High performance liquid chromatography (HPLC) was used to measure plasma TRP and KYN in all participants. The HPLC analyses took place in the laboratory of Dr. Dietmar Fuchs (Innsbruck University, Austria), a lab with established excellent reliability in simultaneous measurement of TRP and KYN by HPLC (35); the interassay coefficient of variation for TPR and KYN are 4.7% and 5.8%, respectively (35).

Statistical analyses

The KYN-TRP ratio was derived for each participant by dividing KYN level by TRP level. The distributions of anti-gliadin IgG, TRP, KYN and KYN-TRP ratio were skewed to the right and a logarithmic transformation was therefore applied, after which the transformed values were used as continuous variables in subsequent analyses. Patients with schizophrenia were categorized as having elevated anti-gliadin IgG or without elevated anti-gliadin IgG, where elevated levels of anti-gliadin IgG were defined as values at the 90th percentile or higher of healthy control participants described in Okusaga et al. (2013), (13) based on a similar method by Dickerson et al. (2010), (2). Independent *t*-tests and chi-square

tests were used to compare continuous and categorical demographic and clinical variables between patients with elevated and those without elevated anti-gliadin IgG. We used independent *t*-tests to compare levels of TRP, KYN and KYN-TRP ratio between patients with elevated anti-gliadin IgG and those without elevated anti-gliadin IgG. Linear regression (adjusting for age, sex, education, body mass index (BMI), total PANSS score, illness duration, antipsychotic medication in chlorpromazine equivalent and ELISA plate) was used to compare TRP, KYN and KYN-TRP ratio between the 2 groups of patients. To evaluate the effects of positive and negative symptoms independently, we also developed two separate linear regression models in which all the previously mentioned potential confounding variables were included as independent variables but instead of total PANSS scores, positive and negative symptoms were included respectively. Partial correlations between anti-gliadin IgG, TRP, KYN and KYN-TRP ratio (adjusting for age, sex, education, BMI, total PANSS score and illness duration) were also evaluated in the patients. Relationships between PANSS scores and TRP, KYN and KYN-TRP ratio were assessed using multiple linear regressions for the total sample of patients and stratified by anti-gliadin IgG antibody status, adjusting for age, sex, education and BMI. All tests were 2-tailed and $p < 0.05$ was considered statistically significant. We have presented geometric means with 95% confidence intervals obtained by exponentiating mean log transformed TRP, KYN and KYN-TRP ratio for adjusted and unadjusted comparisons of the two groups of patients. Statistical analysis was carried out using IBM SPSS version 20 (Armonk, NY: IBM Corp).

Results

Description of the study participants

The demographic and clinical characteristics of the study sample have been described elsewhere (Okusaga et al., 2013). There were no age, sex, BMI, education, or illness duration differences between patients with and those without elevated IgG anti-gliadin antibodies (Table 1). The PANSS positive and negative subscales did not differ between the two patient groups but those without elevated IgG anti-gliadin antibodies had higher scores on the general psychopathology subscale and the total PANSS ($p = 0.044$, $p = 0.011$ respectively).

TRP, KYN and KYN-TRP ratio between patients with and those without elevated anti-gliadin IgG

KYN and KYN-TRP ratio were significantly higher in patients with elevated anti-gliadin IgG antibodies relative to patients without elevated anti-gliadin IgG (geometric mean 2.65 mmol/l, SD = 0.25 vs. 2.25 mmol/l, SD = 0.23, $p < 0.001$ and 0.05, SD = 0.26 vs. 0.04, SD = 0.25, $p = 0.001$ respectively) (Figure 1*).

In the regression model accounting for age, sex, level of education, BMI, total PANSS, illness duration and ELISA plate, KYN and KYN-TRP ratio remained higher in patients with elevated anti-gliadin IgG compared to patients without elevated anti-gliadin IgG (geometric mean difference 1.17 mmol/l, 95% CI 1.07 to 1.28, $p = 0.001$ and 1.16 mmol/l, 95% CI 1.06 to 1.29, $p = 0.002$, respectively). TRP levels did not differ between patients with or without elevated anti-gliadin IgG in unadjusted (59.34 mmol/l, SD = 0.12 vs. 58.89

mmol/l, SD = 0.16, $p = 0.795$) and adjusted analysis (geometric mean difference = 1.00, 95% CI 0.94 to 1.06, $p = 0.994$). These results (for KYN, KYN-TRP ratio and TRP) remained essentially unchanged after post-hoc adjustment for positive and negative symptoms scores.

Correlations between anti-gliadin IgG and TRP, KYN and KYN-TRP ratio

Anti-gliadin IgG correlated with both KYN ($r = 0.12$, $p < 0.001$) and KYN-TRP ratio ($r = 0.11$, $p = 0.002$) and these findings persisted after adjusting for age, sex, level of education, BMI, total PANSS, chlorpromazine equivalent and illness duration. Anti-gliadin IgG did not correlate with TRP in unadjusted ($r = 0.009$, $p = 0.798$) and adjusted analyses.

Associations between PANSS scores and TRP, KYN and KYN-TRP ratio

Overall, PANSS scores were not associated with TRP, KYN or KYN-TRP ratios in the full patient sample ($n = 950$) adjusting for age, sex, sex and education (Table 2). Among those with elevated anti-gliadin IgG, higher KYN-TRP ratios were associated with significantly lower scores on all the PANSS scale scores including Positive, Negative, General and Total scores. Higher KYN was also associated with significantly lowered PANSS total score, only among those with elevated anti-gliadin IgG antibodies.

Discussion

In our sample of 950 patients with schizophrenia, those with elevated IgG antibodies to the wheat protein gliadin also had higher serum levels of KYN relative to those without elevated anti-gliadin IgG. Furthermore, the KYN-TRP ratio, an index of TRP metabolism, was also higher in patients with elevated anti-gliadin IgG. Anti-gliadin IgG levels also positively correlated with both KYN and KYN-TRP ratio. We did not observe any difference in TRP levels between patients with and those without elevated anti-gliadin IgG and there was no significant correlation between anti-gliadin IgG and TRP. To our knowledge this is the first study to investigate anti-gliadin antibody associations with TRP and KYN.

The exact role of anti-gliadin IgG in the pathophysiology of schizophrenia is not fully understood but the result of our study is suggestive of an association of anti-gliadin IgG with immune activation and neuroinflammation now widely accepted to be involved in the illness (14, 15). The presence of anti-gliadin IgG is indicative of gluten sensitivity and since, in our study, antibody levels correlated with KYN and KYN-TRP ratio, one can infer that the severity of sensitivity to gluten in schizophrenia patients might be indicative of the degree of inflammation. A possible explanation for our finding is that patients with schizophrenia who also have elevated anti-gliadin IgG might have a more permeable intestinal mucosa that allows a relatively easier passage of antigenic wheat proteins. As a limitation, we did not measure indicators of leaky gut in our subjects. Indeed patients with schizophrenia have been reported to have intestinal inflammation and the degree of inflammation was observed to correlate with serum levels of anti-gliadin IgG (36). As earlier mentioned, celiac disease has been associated with schizophrenia and increased intestinal mucosa expression of IDO has been reported in patients with celiac disease (37). The increased IDO resulted in elevated KYN and KYN-TRP ratio in celiac disease patients compared to healthy controls (38). We

did not evaluate intestinal IDO in our patient sample therefore we can only speculate that, just as in the case of patients with celiac disease, patients with schizophrenia who have elevated anti-gliadin IgG might also have increased expression of intestinal IDO, which catalyzes the breakdown of TRP into KYN.

From the peripheral circulation, KYN readily crosses the blood-brain barrier where it initiates at least 60% of the KYN pathway metabolism in the brain (38). Once in the brain, KYN becomes involved in two pathways, which are physically segregated in microglial cells and astrocytes respectively (27). The pathway in microglial cells results in the synthesis of QUIN while the pathway in astrocytes results in the synthesis of KYNA. QUIN is an excitatory compound that acts as an agonist at the NMDA glutamate receptor (27). KYNA on the other hand, competitively inhibits all three (NMDA, kainate and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic [AMPA]) ionotropic glutamate receptors (39) and it also inhibits the $\alpha 7$ nicotinic acetylcholine receptor (40). The conversion of KYN to QUIN is initiated by the enzyme kynurenine 3-monooxygenase (KMO) and both KMO gene expression and KMO enzyme activities are reduced in individuals with schizophrenia (41). Since there is a competition between KYNA and QUIN synthesis, the reduced activity of KMO in patients with schizophrenia would be expected to result in increased synthesis of KYNA; this hypothesis has been confirmed in studies that have demonstrated raised levels of KYNA in postmortem brain and CSF of patients with schizophrenia (24–26). It would therefore be expected that in patients with elevated anti-gliadin IgG, increased KYN formation would lead to increased KYNA, in particular in patients with schizophrenia. Increased KYNA in the brain is relevant in the pathophysiology of schizophrenia because by inhibiting glutamate and $\alpha 7$ nicotinic acetylcholine neurotransmission, it can potentially contribute to the symptomatic constellation of the illness (42).

Adding another degree of complexity, the KYN pathway activation has a range of specific immunoregulatory functions, such as induction of regulatory T cell (Treg) differentiation in lymphocyte populations (43). KYN also causes a predominant down regulatory apoptosis of effector T cells, most notably Th1 cells (44, 45). Thus, elevation of KYN in anti-gliadin IgG-positive patients might be keeping the immune activation processes from spiraling out of control, and it could potentially be reducing the immune impact on severity of symptoms. Alternatively, the suppressive effect of high KYN levels might also contribute to reactivation of chronic latent infections previously implicated in schizophrenia (46).

In terms of the relationship between anti-gliadin IgG and the symptoms of schizophrenia, patients with elevated antibodies would be expected to have more severe symptoms based on the reasoning that inflammation is likely to be more pronounced in patients with elevated antibodies (our observed correlation between IgG anti-gliadin and KYN is consistent with this hypothesis). The reasoning that inflammation would be associated with more severe symptoms in schizophrenia is in keeping with clinical trial data supporting the efficacy of anti-inflammatory agents such as cyclooxygenase-2 (COX-2) inhibitors, aspirin and minocycline for schizophrenia symptoms (47, 48). Contrary to our hypothesis, in our sample, positive and negative symptoms did not differ between patients with elevated vs. those without elevated anti-gliadin antibodies, but those without elevated anti-gliadin antibodies had worse general psychopathology and total PANSS scores. In addition, higher

KYN-TRP ratios among patients with elevated anti-gliadin IgG were associated with significantly lowered scores on all the PANSS scale scores including Positive, Negative, General and Total scores. Higher KYN was also associated with significantly lowered PANSS total score, only among those with elevated anti-gliadin IgG antibodies. The mechanisms that would explain the findings of worse symptom scores in patients without elevated anti-gliadin antibodies as well as the inverse relationship between symptom scores and KYN and KYN-TRP ratio are not known to us and we can only speculate that non-linear interactions among inflammatory mediators and anti-gliadin IgG, homeostatic effects rather than mediation, and reciprocal feedback links may be involved. Moreover the differences in general psychopathology and total PANSS between groups, though statistically significant, are small and may not be biologically or clinically relevant. Newly admitted, un-medicated patients with schizophrenia have been found to have elevated interferon- γ , a proinflammatory cytokine that is a strong inducer of IDO, relative to healthy controls (49) and this could account for the elevated KYN and KYN-TRP ratio observed in our study. However, although interferon- γ has been shown to increase release of the human IgG2 subclass of antibody from peripheral blood mononuclear cells, it can decrease release of the IgG1 subclass of antibody (50). The IgG1 subclass of antibody is particularly elevated in coeliac disease, where it is thought to be the primary mediator of antibody-dependent cell-mediated cytotoxicity (ADCC) (51). Thus, the patient group with high overall anti-gliadin IgG could have high interferon- γ , elevated KYN and KYN-TRP ratio, high IgG2, but low IgG1 and therefore low ADCC, with better clinical outcomes. This hypothesis could be tested in future studies. It is noteworthy that a significant cross-reactivity between gliadin and neuronal synapsin has been previously demonstrated (52). However, considering the negative, rather than positive relationship between antibody titers and symptoms of schizophrenia, this mediation by cross-reactivity appears unlikely, and support for the possibility of mediation by a general low grade inflammation, similar to other conditions characterized by a leaky gut, is increased.

The most important limitation of our study are its cross-sectional design, not measuring molecular or cellular mediators of inflammation, and all schizophrenia participants being on antipsychotic medication. We only measured TRP and KYN peripherally and did not evaluate levels in the CSF, nor did we measure KYNA, a putative mediator in schizophrenia. We did not account for the time of the day of the blood draw, although our previous research did not identify significant diurnal variation in KYN in clinical samples (unpublished observation). We also did not measure the subclasses of anti-gliadin IgG antibodies. Since IgG subclasses differ in their ability to mediate effector responses, this could provide additional mechanistic insights into the involvement of anti-gliadin antibodies in the pathophysiology of schizophrenia (53). In the absence of inflammatory markers, we cannot affirm that the elevation in KYN resulted from IDO activation. An alternative explanation could be that individuals with anti-gliadin IgG positivity are more vulnerable to stress and, through increased actions of cortisol on TDO, respond with the identical outcome of elevation of KYN and reduction in TRP. Also, individuals with less severe symptoms (and potentially a better insight) may experience a higher level of stress, and thus a higher level of KYN production, predominantly through TDO activation. Taking all of our observations into account, anti-gliadin antibodies may predispose to leaky gut/gut inflammation and

subsequent low grade increases in systemic inflammation, which may activate IDO, leading to increased KYN and ultimately increased KYNA in the central nervous system (CNS), which would then antagonize NMDA receptors (and have other effects on glutamate), implicated in the neurobiology of schizophrenia. A limitation to address in future studies is analyzing and accounting for measures of leaky gut as a potential mediator of our reported associations.

Strengths of this study include the relatively large sample, confirmation of schizophrenia diagnosis by the gold standard method (Structured Clinical Interview for DSM-IV) (33) and adjustment for many potential confounders (such as antipsychotic medication dosage, symptom scores) in the data analysis.

In conclusion, we are reporting a link between elevated serum anti-gliadin IgG levels and elevated serum KYN and KYN-TRP ratio. Future clinical trials of gluten-free diet in individuals with schizophrenia with elevated anti-gliadin IgG are warranted as such trials could shed more light on the association of wheat protein, antibodies to wheat protein, inflammation and molecules, such as KYN, connecting inflammation with brain structure and function. This in the long run may contribute to discovering and testing novel prophylactic and therapeutic interventions.

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List of abbreviations

ADCC	antibody-dependent cell-mediated cytotoxicity
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BMI	body mass index
COX-2	cyclooxygenase-2
CNS	central nervous system
CSF	cerebrospinal fluid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
EDTA	Ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay

HPLC	High performance liquid chromatography
IDO	indoleamine 2,3-dioxygenase
IgA	immunoglobulin AIgG, immunoglobulin G
KMO	kynurenine 3-monooxygenase
KYN	kynurenine
KYNA	kynurenic acid
NMDA	N-methyl-D-aspartic acid
PANSS	Positive and Negative Syndrome Scale
QUIN	quinolinic acid
TDO	tryptophan 2,3-dioxygenase
TRP	tryptophan

References

1. Cascella NG, Kryszak D, Bhatti B, Gregory P, Kelly DL, Mc Evoy JP, Fasano A, Eaton WW. Prevalence of celiac disease and gluten sensitivity in the United States clinical antipsychotic trials of intervention effectiveness study population. *Schizophr Bull.* 2011; 37:94–100. [PubMed: 19494248]
2. Dickerson F, Stallings C, Origoni A, Vaughan C, Khushalani S, Leister F, Yang S, Krivogorsky B, Alaedini A, Yolken R. Markers of gluten sensitivity and celiac disease in recent-onset psychosis and multi-episode schizophrenia. *Biol Psychiatry.* 2010; 68:100–4. [PubMed: 20471632]
3. Okusaga O, Yolken RH, Langenberg P, Sleemi A, Kelly DL, Vaswani D, Giegling I, Hartmann AM, Konte B, Friedl M, Mohyuddin F, Groer MW, Rujescu D, Postolache TT. Elevated gliadin antibody levels in individuals with schizophrenia. *World J Biol Psychiatry.* 2013; 14:509–15. [PubMed: 23282016]
4. Reichelt KL, Landmark J. Specific IgA antibody increases in schizophrenia. *Biol Psychiatry.* 1995; 37:410–3. [PubMed: 7772650]
5. Karlsson H, Blomstrom A, Wicks S, Yang S, Yolken RH, Dalman C. Maternal antibodies to dietary antigens and risk for nonaffective psychosis in offspring. *Am J Psychiatry.* 2012; 169:625–32. [PubMed: 22535227]
6. Eaton W, Mortensen PB, Agerbo E, Byrne M, Mors O, Ewald H. Coeliac disease and schizophrenia: population based case control study with linkage of Danish national registers. *BMJ.* 2004; 328:438–9. [PubMed: 14976100]
7. Kalaydjian AE, Eaton W, Cascella N, Fasano A. The gluten connection: the association between schizophrenia and celiac disease. *Acta Psychiatr Scand.* 2006; 113:82–90. [PubMed: 16423158]
8. Ludvigsson JF, Osby U, Ekblom A, Montgomery SM. Coeliac disease and risk of schizophrenia and other psychosis: a general population cohort study. *Scand J Gastroenterol.* 2007; 42:179–85. [PubMed: 17327937]
9. Samaroo D, Dickerson F, Kasarda DD, Green PH, Briani C, Yolken RH, Alaedini A. Novel immune response to gluten in individuals with schizophrenia. *Schizophr Res.* 2010; 118:248–55. [PubMed: 19748229]
10. Volta U, Caio G, De Giorgio R, Henriksen C, Skodje G, Lundin KE. Non-celiac gluten sensitivity: A work-in-progress entity in the spectrum of wheat-related disorders. *Best Pract Res Clin Gastroenterol.* 2015; 29:477–91. [PubMed: 26060112]

11. Daulatzai MA. Non-celiac gluten sensitivity triggers gut dysbiosis, neuroinflammation, gut-brain axis dysfunction, and vulnerability for dementia. *CNS Neurol Disord Drug Targets*. 2015; 14:110–31. [PubMed: 25642988]
12. Porcelli B, Verdino V, Bossini L, Terzuoli L, Fagiolini A. Celiac and non-celiac gluten sensitivity: a review on the association with schizophrenia and mood disorders. *Auto Immun Highlights*. 2014; 5:55–61. [PubMed: 26000156]
13. Jackson J, Eaton W, Cascella N, Fasano A, Santora D, Sullivan K, Feldman S, Raley H, McMahon RP, Carpenter WT Jr, Demyanovich H, Kelly DL. Gluten sensitivity and relationship to psychiatric symptoms in people with schizophrenia. *Schizophr Res*. 2014; 159:539–42. [PubMed: 25311778]
14. Najjar S, Pearlman DM. Neuroinflammation and white matter pathology in schizophrenia: systematic review. *Schizophr Res*. 2015; 161:102–12. [PubMed: 24948485]
15. Girgis RR, Kumar SS, Brown AS. The cytokine model of schizophrenia: emerging therapeutic strategies. *Biol Psychiatry*. 2014; 75:292–9. [PubMed: 24439555]
16. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. *Biol Psychiatry*. 2011; 70:663–71. [PubMed: 21641581]
17. Brown AS. The Kraepelinian Dichotomy From the Perspective of Prenatal Infectious and Immunologic Insults. *Schizophr Bull*. 2015
18. Brown JS Jr. Celiac disease and schizophrenia. *Am J Psychiatry*. 2012; 169:991–2.
19. Benros ME, Pedersen MG, Rasmussen H, Eaton WW, Nordentoft M, Mortensen PB. A nationwide study on the risk of autoimmune diseases in individuals with a personal or a family history of schizophrenia and related psychosis. *Am J Psychiatry*. 2014; 171:218–26. [PubMed: 24129899]
20. McAllister AK. Major histocompatibility complex I in brain development and schizophrenia. *Biol Psychiatry*. 2014; 75:262–8. [PubMed: 24199663]
21. Kagnoff MF. Two genetic loci control the murine immune response to A-gliadin, a wheat protein that activates coeliac sprue. *Nature*. 1982; 296:158–60. [PubMed: 6801524]
22. Bumb JM, Enning F, Leweke FM. Drug repurposing and emerging adjunctive treatments for schizophrenia. *Expert Opin Pharmacother*. 2015; 16:1049–67. [PubMed: 25866122]
23. Linderholm KR, Skogh E, Olsson SK, Dahl ML, Holtze M, Engberg G, Samuelsson M, Erhardt S. Increased levels of kynurenine and kynurenic acid in the CSF of patients with schizophrenia. *Schizophr Bull*. 2012; 38:426–32. [PubMed: 20729465]
24. Schwarcz R, Rassoulpour A, Wu HQ, Medoff D, Tamminga CA, Roberts RC. Increased cortical kynurenate content in schizophrenia. *Biol Psychiatry*. 2001; 50:521–30. [PubMed: 11600105]
25. Erhardt S, Blennow K, Nordin C, Skogh E, Lindstrom LH, Engberg G. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neurosci Lett*. 2001; 313:96–8. [PubMed: 11684348]
26. Sathyaikumar KV, Stachowski EK, Wonodi I, Roberts RC, Rassoulpour A, McMahon RP, Schwarcz R. Impaired kynurenine pathway metabolism in the prefrontal cortex of individuals with schizophrenia. *Schizophr Bull*. 2011; 37:1147–56. [PubMed: 21036897]
27. Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. *Nat Rev Neurosci*. 2012; 13:465–77. [PubMed: 22678511]
28. Steeds H, Carhart-Harris RL, Stone JM. Drug models of schizophrenia. *Ther Adv Psychopharmacol*. 2015; 5:43–58. [PubMed: 25653831]
29. Poels EM, Kegeles LS, Kantrowitz JT, Slifstein M, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis RR. Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. *Mol Psychiatry*. 2014; 19:20–9. [PubMed: 24166406]
30. Erhardt S, Schwieler L, Nilsson L, Linderholm K, Engberg G. The kynurenic acid hypothesis of schizophrenia. *Physiol Behav*. 2007; 92:203–9. [PubMed: 17573079]
31. Sublette ME, Postolache TT. Neuroinflammation and depression: the role of indoleamine 2,3-dioxygenase (IDO) as a molecular pathway. *Psychosom Med*. 2012; 74:668–72. [PubMed: 22923699]
32. Babinkostova Z, Stefanovski B, Janicevic-Ivanovska D, Samardziska V, Stojanovska L. Serum cortisol and DHEA-S levels in schizophrenic patients with different response to antipsychotic therapy: association with psychopathology. *Prilozi*. 2015; 36:175–83. [PubMed: 26076787]

33. First, M., Spitzer, RL., Gibbon, M., Williams, JBW. Research Version, Patient Edition. New York: Biometrics Research, New York State Psychiatric Institute; 2002. Structured Clinical Interview for DSM-IV-TR Axis I Disorders.
34. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull.* 1987; 13:261–76. [PubMed: 3616518]
35. Widner B, Werner ER, Schennach H, Wachter H, Fuchs D. Simultaneous measurement of serum tryptophan and kynurenine by HPLC. *Clin Chem.* 1997; 43:2424–6. [PubMed: 9439467]
36. Severance EG, Yolken RH, Eaton WW. Autoimmune diseases, gastrointestinal disorders and the microbiome in schizophrenia: more than a gut feeling. *Schizophr Res.* 2014
37. Torres MI, Lopez-Casado MA, Lorite P, Rios A. Tryptophan metabolism and indoleamine 2,3-dioxygenase expression in coeliac disease. *Clin Exp Immunol.* 2007; 148:419–24. [PubMed: 17362267]
38. Gal EM, Sherman AD. L-kynurenine: its synthesis and possible regulatory function in brain. *Neurochem Res.* 1980; 5:223–39. [PubMed: 6154900]
39. Perkins MN, Stone TW. An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. *Brain Res.* 1982; 247:184–7. [PubMed: 6215086]
40. Wu HQ, Pereira EF, Bruno JP, Pellicciari R, Albuquerque EX, Schwarcz R. The astrocyte-derived alpha7 nicotinic receptor antagonist kynurenic acid controls extracellular glutamate levels in the prefrontal cortex. *J Mol Neurosci.* 2010; 40:204–10. [PubMed: 19690987]
41. Wonodi I, Stine OC, Sathyaikumar KV, Roberts RC, Mitchell BD, Hong LE, Kajii Y, Thaker GK, Schwarcz R. Downregulated kynurenine 3-monooxygenase gene expression and enzyme activity in schizophrenia and genetic association with schizophrenia endophenotypes. *Arch Gen Psychiatry.* 2011; 68:665–74. [PubMed: 21727251]
42. Javitt DC. Twenty-five years of glutamate in schizophrenia: are we there yet? *Schizophr Bull.* 2012; 38:911–3. [PubMed: 22987849]
43. Fallarino F, Grohmann U, You S, McGrath BC, Cavener DR, Vacca C, Orabona C, Bianchi R, Belladonna ML, Volpi C, Santamaria P, Fioretti MC, Puccetti P. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. *J Immunol.* 2006; 176:6752–61. [PubMed: 16709834]
44. Bauer TM, Jiga LP, Chuang JJ, Randazzo M, Opelz G, Terness P. Studying the immunosuppressive role of indoleamine 2,3-dioxygenase: tryptophan metabolites suppress rat allogeneic T-cell responses in vitro and in vivo. *Transpl Int.* 2005; 18:95–100. [PubMed: 15612990]
45. Mellor AL, Munn D, Chandler P, Keskin D, Johnson T, Marshall B, Jhaver K, Baban B. Tryptophan catabolism and T cell responses. *Adv Exp Med Biol.* 2003; 527:27–35. [PubMed: 15206713]
46. Torrey EF, Bartko JJ, Lun ZR, Yolken RH. Antibodies to *Toxoplasma gondii* in patients with schizophrenia: a meta-analysis. *Schizophr Bull.* 2007; 33:729–36. [PubMed: 17085743]
47. Keller WR, Kum LM, Wehring HJ, Koola MM, Buchanan RW, Kelly DL. A review of anti-inflammatory agents for symptoms of schizophrenia. *J Psychopharmacol.* 2013; 27:337–42. [PubMed: 23151612]
48. Muller N, Myint AM, Krause D, Weidinger E, Schwarz MJ. Anti-inflammatory treatment in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry.* 2013; 42:146–53. [PubMed: 23178230]
49. Kim YK, Myint AM, Lee BH, Han CS, Lee HJ, Kim DJ, Leonard BE. Th1, Th2 and Th3 cytokine alteration in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry.* 2004; 28:1129–34. [PubMed: 15610925]
50. Kawano Y, Noma T, Yata J. Regulation of human IgG subclass production by cytokines. IFN-gamma and IL-6 act antagonistically in the induction of human IgG1 but additively in the induction of IgG2. *J Immunol.* 1994; 153:4948–58. [PubMed: 7963558]
51. Saalman R, Dahlgren UI, Fallstrom SP, Hanson LA, Ahlstedt S, Wold AE. IgG subclass profile of serum antigliadin antibodies and antibody-dependent cell-mediated cytotoxicity in young children with coeliac disease. *Scand J Immunol.* 2001; 53:92–8. [PubMed: 11169212]

52. Alaedini A, Okamoto H, Briani C, Wollenberg K, Shill HA, Bushara KO, Sander HW, Green PH, Hallett M, Latov N. Immune cross-reactivity in celiac disease: anti-gliadin antibodies bind to neuronal synapsin I. *J Immunol.* 2007; 178:6590–5. [PubMed: 17475890]
53. Nimmerjahn F, Ravetch JV. Divergent immunoglobulin g subclass activity through selective Fc receptor binding. *Science.* 2005; 310:1510–2. [PubMed: 16322460]

Figure 1.

Serum kynurenine (KYN) concentrations (mmol/l) (a), kynurenine-tryptophan (KYN/TRP) ratio (b) and serum tryptophan (TRP) concentrations (mmol/l) (c) in patients with schizophrenia with and without elevated anti-gliadin IgG. Error bars are representing 95% confidence intervals, and the little circle stands for the mean of the log-transformed biomarker. Elevated levels of anti-gliadin IgG were defined as values at the 90th percentile or higher of healthy control participants described in Okusaga et al. (2013) Serum KYN and TRP concentration and KYN-TRP ratio were Log-transformed to normalize the data.

*Geometric means reported in the text were calculated by exponentiating mean log transformed TRP, KYN and KYN-TRP ratio for adjusted and unadjusted comparisons of the two groups of patients.

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Table 1

Demographic and clinical characteristics of patients with schizophrenia dichotomized into those with elevated and those without elevated anti gliadin titers.

Characteristics	Patients with schizophrenia with elevated anti gliadin titers (n = 208)	Patients with schizophrenia without elevated anti gliadin titers (n =742)	p-value*
Age, years (mean $\pm$ SD)	36.9 $\pm$ 11.3	38.2 $\pm$ 11.7	0.142
Gender male, n (%)	132 (63.5)	468 (63.1)	0.918
Education Level (n, %)			0.426
Primary	97 (46.6)	308 (41.6)	
Secondary	49 (23.6)	192 (25.9)	
Tertiary	62 (29.8)	242 (32.5)	
BMI, kg/m ² (mean $\pm$ SD)	27.0 $\pm$ 5.2	27.1 $\pm$ 5.6	0.831
Duration of illness, months (mean $\pm$ SD)	12.2 $\pm$ 15.4	13.5 $\pm$ 16.5	0.322
PANSS (mean $\pm$ SD)			
Positive symptoms	27.3 $\pm$ 6.2	27.8 $\pm$ 6.4	0.268
Negative symptoms	23.5 $\pm$ 8.2	24.6 $\pm$ 7.3	0.089
Total score	98.0 $\pm$ 20.8	102.3 $\pm$ 21.6	0.011

Table 2

Relationship between PANSS scores and measures of kynurenine metabolism showing adjusted standardized coefficients (beta) from multiple linear regression models.

	Schizophrenia patients with elevated anti gliadin IgG titers (n = 208)	Schizophrenia patients without elevated anti gliadin IgG titers (n = 742)	All Schizophrenia patients (n = 950)
PANSS Scale Scores			
Positive symptoms	Beta ^a	Beta ^a	Beta ^a
KYN-TRP ratio	-0.184 *	0.031	-0.022
KYN	-0.137	0.020	-0.018
TRP	0.103	-0.019	0.008
Negative symptoms			
KYN-TRP ratio	-0.168 *	-0.025	-0.069
KYN	-0.136	0.002	-0.043
TRP	0.071	0.040	0.047
General psychopathology			
KYN-TRP ratio	-0.181 *	0.053	-0.008
KYN	-0.131	0.045	-0.004
TRP	0.111	-0.018	0.007
PANSS Total Score			
KYN-TRP ratio	-0.211 **	0.030	-0.035
KYN	-0.159 *	0.032	-0.023
TRP	0.114	-0.002	0.023

* p < .05;

** p < .01

^a Standardized beta coefficients derived from separate multiple linear regression models predicting each PANSS scale score for each measure of kynurenine metabolism (KYN-TRP ratio, KYN, TRP) adjusted for BMI, age, education and sex.

PANSS = Positive and Negative Syndrome Scale

KYN = kynurenine (log transformed)

TRP = tryptophan (log transformed)